



(43) International Publication Date
3 June 2004 (03.06.2004)

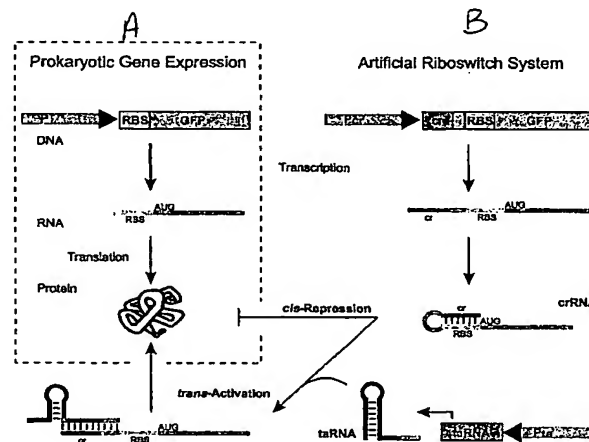
PCT

(10) International Publication Number
WO 2004/046321 A2

- (51) International Patent Classification⁷: **C12N**
- (21) International Application Number:
PCT/US2003/036506
- (22) International Filing Date:
14 November 2003 (14.11.2003)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
60/426,891 15 November 2002 (15.11.2002) US
- (71) Applicant (for all designated States except US):
TRUSTEES OF BOSTON UNIVERSITY [US/US];
One Sherborn Street, Boston, MA 02215 (US).
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): **COLLINS, James, J. [US/US];** 118 Glen Avenue, Newton, MA 02459 (US). **ISAACS, Farren, J. [US/US];** 110 Babcock Street, #17, Brookline, MA 02446 (US). **DWYER, Daniel, J. [US/US];** 8 Naples Road, Brookline, MA 02446 (US). **CANTOR,**
- (74) Agent: **GERBER, Monica, R.;** Choate, Hall & Stewart, 53 State Street, Exchange Place, Boston, MA 02109 (US).
- (81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.
- (84) Designated States (*regional*): ARIPO patent (BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).
- Published:
— without international search report and to be republished upon receipt of that report

[Continued on next page]

(54) Title: **CIS/TRANS RIBOREGULATORS**



(57) Abstract: The present invention provides nucleic acid molecules, DNA constructs, plasmids, and methods for post-transcriptional regulation of gene expression using RNA molecules to both repress and activate translation of an open reading frame. Repression of gene expression is achieved through the presence of a regulatory nucleic acid element (the cis-repressive RNA or crRNA) within the 5' untranslated region (5' UTR) of an mRNA molecule. The nucleic acid element forms a hairpin (stem/loop) structure through complementary base pairing. The hairpin blocks access to the mRNA transcript by the ribosome, thereby preventing translation. In particular, in embodiments of the invention designed to operate in prokaryotic cells, the stem of the hairpin secondary structure sequesters the ribosome binding site (RBS). In embodiments of the invention designed to operate in eukaryotic cells, the stem of the hairpin is positioned upstream of the start codon, anywhere within the 5' UTR of an mRNA. A small RNA (trans-activating RNA, or taRNA), expressed in trans, interacts with the crRNA and alters the hairpin structure. This alteration allows the ribosome to gain access to the region of the transcript upstream of the start codon, thereby activating translation from its previously repressed state.

W0004/046321 A2



For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.